The effect of nicotine on sensorimotor gating is modulated by a CHRNA3 polymorphism

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Abstract: RATIONALE: Prepulse inhibition (PPI) of the acoustic startle response, a measure of sensorimotor gating, can be enhanced by nicotine. Moreover, the TT genotype of the nicotinic acetylcholine receptor (nAChR) 3-subunit (CHRNA3) rs1051730 polymorphism has previously been associated with diminished PPI and nicotine dependence. OBJECTIVES: We tested whether this CHRNA3 polymorphism also modulates the nicotine-induced enhancement of PPI. METHODS: We assessed the effect of nicotine on PPI, startle reactivity, and habituation in 52 healthy nonsmoking volunteers genotyped for CHRNA3 rs1051730 in a double-blind, placebo-controlled, counterbalanced, within-subjects design. Additionally, cotinine plasma levels were measured. RESULTS: Nicotine significantly enhanced PPI in TT homozygotes only and tended to worsen PPI in TC and CC carriers. Additionally, nicotine significantly reduced startle habituation. CONCLUSIONS: The present findings imply that the effect of nicotine on sensorimotor gating is modulated by nAChR 3-subunits. Thus, genetic variation in nicotinic receptor genes might be an important connecting link between early attentional processes and smoking behavior.

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The effect of nicotine on sensorimotor gating is modulated by a *CHRNA3* polymorphism

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Abstract

Rationale Prepulse inhibition (PPI) of the acoustic startle response, a measure of sensorimotor gating, can be enhanced by nicotine. Moreover, the TT-genotype of the nicotinic acetylcholine receptor (nAChR) α3-subunit (*CHRNA3*) rs1051730 polymorphism has previously been associated with diminished PPI and nicotine dependence. *Objectives* We tested whether this *CHRNA3* polymorphism also modulates the nicotine-induced enhancement of PPI. *Methods* We assessed the effect of nicotine on PPI, startle reactivity, and habituation in 52 healthy non-smoking volunteers genotyped for *CHRNA3* rs1051730 in a double-blind, placebo-controlled, counterbalanced, within-subjects design. Additionally, cotinine plasma levels were measured. *Results* Nicotine significantly enhanced PPI in TT-homozygotes only and tended to worsen PPI in TC- and CC-carriers. Additionally, nicotine significantly reduced startle habituation. *Conclusions* The present findings imply that the effect of nicotine on sensorimotor gating is modulated by nAChR α3-subunits. Thus, genetic variation in nicotinic receptor genes might be an important connecting link between early attentional processes and smoking behavior.

Keywords

Prepulse inhibition, sensorimotor gating, *CHRNA3*, nicotine, nicotinic acetylcholine receptor, rs1051730

Introduction

In humans, prepulse inhibition (PPI) is commonly measured as PPI of the acoustic startle reflex: a relatively weak and non-startling noise (the prepulse) is presented 30-500 ms before a strong startle-eliciting sound (the pulse) resulting in a reduction of the eye-blink startle reflex (Graham 1975). More specifically, PPI is thought to reflect an automatic filtering process in which the processing of the prepulse is protected from disruption which leads to attenuated processing of the pulse (Baschnagel and Hawk 2008; Graham et al. 1975). PPI is commonly viewed as an operational measure of a process called "sensorimotor gating," by which excess or trivial stimuli are screened or "gated out" of awareness so that an individual can focus attention on the most salient aspects of the stimulus-laden environment (Braff et al. 2001).

Nicotine, an agonist of nicotinic acetylcholine receptors (nAChR), consistently enhances PPI in humans (Baschnagel and Hawk 2008; Della Casa et al. 1998; Duncan et al. 2001; Kumari et al. 1996; Kumari et al. 1997) and animals (Acri et al. 1995; Acri et al. 1994; Curzon et al. 1994; Faraday et al. 1999; Schreiber et al. 2002; Spielewoy and Markou 2004). The exact mechanism by which nicotine enhances PPI is not yet understood but one way nicotine may improve PPI is by enhancing the diminishment of the processing of the pulse suggesting that nicotine may improve stimulus filtering (Baschnagl and Hawk 2008). In mice, the PPI-enhancing effect of nicotine is strain dependent (Faraday et al. 1999) suggesting genetic influences in nicotine-induced modulation of PPI (Kumari and Postma 2005).

In a recent study by our group, it was demonstrated that variants in *CHRNA3*, the gene encoding for the neuronal acetylcholine receptor subunit α3, are associated with PPI (Petrovsky et al. 2010). More precisely, we showed that two common and strongly linked *CHRNA3* single nucleotide polymorphisms (rs1051730/rs1317286) were associated with PPI in two independent samples of healthy human volunteers and patients with schizophrenia (Petrovsky et al. 2010). The TT/GG genotype of rs1051730/rs1317286 was associated with

decreased PPI levels in both groups (Petrovsky et al. 2010). Our findings from that study further support the view that the cholinergic system plays a key role in pre-attentional and attentional mechanisms (Bosch and Schmid 2008; Heishman et al. 2010; Hong et al. 2008). Moreover, our previous results indicate that there might be shared variance in the molecular genetic substrates for both nicotine dependence and sensorimotor gating: interestingly, the *CHRNA3* rs1051730 T allele and the rs1317286 G allele have been firmly established as risk alleles for nicotine dependence (Berrettini et al. 2008; Bierut et al. 2008; Caporaso et al. 2009; Saccone et al. 2009). Probably, a genetically induced alteration in the nAChR-system is (in part) responsible for both deficits in sensorimotor gating and increased risk for smoking.

In a recent clinical trial, the α4β2 nAChR agonist varenicline did not improve PPI in a sample of smoking and non-smoking patients with schizophrenia or schizoaffective disorder (Hong et al. 2011a). However, varenicline reduced startle reactivity regardless of smoking status (Hong et al. 2011a). The notion that the α4β2 nAChR agonist varenicline did not enhance PPI speaks in favor of the idea that PPI is mainly modulated at the α3 nAChR subtype, possibly at the α3β2 or the α3β4 receptors.

Assuming that individuals with an altered nAChR-system are those individuals who exhibit sensorimotor gating deficits and an increased risk for smoking/nicotine dependence, it might be the case that these individuals use nicotine in order to self-medicate PPI deficits (Kumari and Postma 2005). In particular, there is considerable empirical support for the idea that smoking in schizophrenia may represent an attempt to self-medicate some of the neurocognitive deficits of this disorder, including PPI (Kumari and Postma 2005).

Therefore, it would be interesting to test with a pharmacogenetic design whether individuals carrying cholinergic variants associated with both diminished PPI and nicotine dependence (as it is the case with *CHRNA3* rs1051730/rs1317286) display enhanced PPI under nicotine application. To date, there is one published study providing evidence that dopaminergic polymorphisms might influence the effect of nicotine on PPI in humans

(Perkins et al. 2008). However, there are no published pharmacogenetic studies on the modulation of nicotine effects by cholinergic polymorphisms regarding PPI, neither in animals, nor in humans.

Thus, based on prior findings on the nicotine-induced enhancement of PPI, the finding that the *CHRNA3* rs1051730 T-allele has been established as a risk allele for nicotine dependence, and based on our previous result that diminished PPI is associated with the homozygous *CHRNA3* rs1051730 TT genotype, we tested the hypothesis that nicotine specifically enhances PPI in *CHRNA3* rs1051730 TT carriers. We restricted the present study to healthy individuals as this allows the study of pharmacogenetic interactions in the absence of clinical and treatment confounds. Finally, we assessed cotinine plasma levels to control for individual differences in nicotine uptake from the patches.

Materials and Methods

Participants

Healthy volunteers were recruited through local advertisement and by contacting a random sample of the inhabitants of Bonn based on a list from the city registry. Non-smoking individuals aged between 18 and 55 years were included in the present study. Non-smokers were defined as individuals who had smoked no more than 100 cigarettes during their lifetime and had not smoked a cigarette in the past year. All participants were screened with the Structured Clinical Interview for DSM-IV (SCID-I, German version; Wittchen et al. 1997) to exclude participants with a current or lifetime Axis I disorder. Further exclusion criteria included head injury with loss of consciousness of >5 min, lifetime history of alcohol or substance abuse or dependence, a history of neurological illness or another severe medical condition, a relative with psychosis, severe obesity (body mass index BMI >35), and un-corrected visual impairments and hearing impairments.

Furthermore, the following exclusion criteria were employed in order to avoid serious side effects caused by the nicotine application: cardiovascular disease, hypertension, atopic or eczematous dermatitis (due to localised patch sensitivity), severe renal or hepatic impairment or active peptic ulcers, hyperthyroidism, pheochromocytoma, insulin-dependent diabetes, hypersensitivity to patches, hypersensitivity to nicotine, or any of the excipients of the patches.

Approval of the local ethics committee and the German Federal Institute for Drugs and Medical Devices (BfArM) was obtained and the study was registered with http://www.clinicaltrials.gov (ClinicalTrials.gov Identifier: NCT01315002). Participants provided written informed consent before inclusion. All subjects were compensated for their participation.

Procedures

A study physician ensured that all inclusion and exclusion criteria were met. On both testing days, a urine drug screening test (nal von minden, Moers, Germany) was applied before patch application to ensure subjects had abstained from amphetamine, benzodiazepine, cocaine, cannabinoides, and opiates. All female subjects additionally underwent a urine pregnancy test (Hitado hCG, Hitado Direkt, Endingen, Germany) on both testing days to confirm they were not pregnant.

Nicotine was administered in a double-blind, placebo-controlled, repeated-measures design. Each subject completed two startle response testing sessions (one nicotine session and one placebo session, the order of sessions was counterbalanced). Nicotine was applied via patches (NiQuitin Clear, GlaxoSmithKline, 7mg nicotine patch). Placebo patches (Fink and Walter GmbH, Germany) of similar appearance were applied. Both patches were applied non-visibly to the upper back of the subject by a research assistant who was not running the test sessions in order to ensure double-blindness. Acoustic startle measurement commenced three

hours after patch application. Nicotine administration using the NiQuitin patch generates a fast-rising nicotine plasma level (a nicotine plateau level is achieved after 2 to 4h after application according to the Summary of Product Characteristics of NiQuitin Clear). The nicotine doses were in accordance with already published studies which demonstrated nicotine effects on cognitive functions (Depatie et al. 2002; Levin et al. 1998; Petrovsky et al. 2012; Poltavski and Petros 2006). Table 1 illustrates the experimental procedures of the two testing sessions.

(Table 1 about here)

Cotinine plasma levels

Blood samples were collected immediately after the startle measurements in anticoagulant EDTA 9ml tubes and centrifuged at 3000rpm for 10min. Plasma was collected, allotted to two aliquots, and frozen at -80°C in order to be later analyzed for cotinine. Cotinine was quantified with liquid chromatography-mass spectromety (LC-MS), a highly specific and sensitive method (Gabr et al. 2011).

Genotyping

The *CHRNA3* rs1051730 single nucleotide polymorphism (SNP) was analyzed by a TaqMan assay (Applied Biosystems, Foster City, California). The procedure has been described in detail elsewhere (Petrovsky et al. 2010). Genotyping of participants was performed after they had completed the two startle response testing sessions.

Startle Response Measurement

Each examination began with a 2-min acclimation period of 70-dB background noise that was continued throughout the session. Participants received 61 white-noise sound pulses at an intensity of 115 dB (duration of 40 ms) separated by variable inter-trial intervals (ITIs)

between 9 and 17 s (mean=13 s). In 40 of the trials, the pulse was preceded by a 20-ms, 85-dB white-noise prepulse with stimulus-onset asynchronies (SOAs) of 30, 60, 120 and 240 ms (10 trials each). Additionally, participants received 10 no-stimulus-trials in which background noise continued and 10 prepulse-alone-trials. Overall, the session consisted of 81 trials. The initial trial was a pulse-alone (PA) trial, which was separated for further analysis. All following trials were presented in a pseudo-randomized order. The entire test session lasted approximately 20 min. Trial exclusion and scoring criteria were identical to those used in previous studies (Quednow et al. 2006a; Quednow et al. 2006b). Subjects with response rejections >50% were excluded from data analysis.

Statistical analyses

Startle reactivity was assessed by the mean amplitude of the first block of PA trials and the mean amplitude of all PA trials. The mean percent PPI of startle magnitude was calculated using the formula [% PPI = 100 × (magnitude on pulse alone (PA) trials - magnitude on prepulse (PP) trials)/magnitude on PA trials] (Quednow et al. 2006a; Quednow et al. 2006b). For the assessment of startle habituation, PA trials were divided each into four blocks. PA trials from the first, second, and third block were taken to calculate PPI (Quednow et al. 2006a). The percentage early habituation was calculated as the reduction in startle magnitude between the first and second block of PA trials [%HAB=100×(first block–second block)/first block]. The percentage total habituation was calculated as the reduction in startle magnitude between the first and last block (i.e., the fourth block) of PA trials [%HAB=100×(first block–last block)/first block]. Linear gradient coefficient *b* was calculated as a further measure of habituation: [$b=(n\sum xy-(\sum x)(\sum y))/(n\sum x^2-(\sum x)^2)$; x=block number, y=startle amplitude PA trial per block].

All demographic data and psychophysiological parameters were analyzed by analysis of variance (ANOVA) with the exception of frequency data. Frequency data were analyzed

using χ^2 tests. Startle reactivity and habituation were analyzed with repeated measures analysis of variance (ANOVA) with drug (placebo, nicotine) as within-subjects factor and *CHRNA3* rs1051730 genotype (TT,TC,CC) and order (placebo/nicotine, nicotine/placebo) as between-subjects factors. PPI data were analyzed with repeated measures analysis of covariance (ANCOVA) with drug (placebo, nicotine) and SOA (30,60,120,240 ms) as within-subjects factors and *CHRNA3* rs1051730 genotype (TT,TC,CC) and order (placebo/nicotine, nicotine/placebo) as between-subjects factors as well as sex as a covariate given that sex is known to affect PPI (Swerdlow et al. 1997), and cotinine plasma level as another covariate to control for inter-individual differences in the metabolism of nicotine. A paired two-sample t-test was used to test whether cotinine plasma levels differed between the placebo session and the nicotine session (serving as a manipulation check). We also explored whether PPI change scores (i.e., difference values: placebo data–nicotine data) were correlated with cotinine plasma level using Pearson's correlations. Greenhouse-Geisser correction of p-values was applied when sphericity was violated. P-values of post-hoc tests were Bonferroni-corrected. The significance level of all statistical tests was set at $p<.05$.

Results

Psychophysiological parameters

Acoustic startle data from 58 subjects were obtained. Out of these 58 subjects, 3 quit the study due to nausea caused by the nicotine application. Out of the remaining 55 subjects, 3 were identified as startle-non-responders (5.5%) and were excluded from further analyses. The proportion of non-responders in our sample is in accordance with observations in other samples of healthy volunteers (Blumenthal et al. 2005). Thus, the final sample included 52 subjects. The rs1051730 genotype groups did not differ regarding age, education, body mass index, and sex distribution (see Table 2). Genotype frequencies were distributed in accordance with Hardy-Weinberg equilibrium (HWE)($\chi^2(1)=0.15$, $p=.69$). Genotype groups

did not differ regarding early habituation, and total habituation. Confirming our earlier results, PPI was considerably lower in TT-allele carriers compared to CC-allele carriers with a slightly smaller effect size (d=0.55) as previously reported for healthy subjects (d=0.79) (Petrovsky et al. 2010). However, due to the smaller sample and effect size, the overall genotype effect was not significant. Also startle reactivity (assessed by the mean amplitude of the first block of PA trials) tended to be different between genotype groups with (p=.07, see Table 2). Genotype groups differed significantly in habituation slope *b* ($F(2,49)=4.52$, p=.02, $\eta_p^2=.16$)(see Figure 1 and Table 2). Post-hoc testing revealed that TT-carriers did not significantly differ from TC-carriers in this habituation coefficient (p=1.00). However, TT-carriers exhibited a significantly decreased habituation coefficient compared to CC-carriers (p=.03). TC-carriers tended to show a decreased habituation coefficient compared to CC-carriers (p=.055).

(Table 2 about here)

(Figure 1 about here)

Effects of nicotine on startle reactivity and habituation

For startle reactivity and habituation measures there were neither main effects of order (all p>.36) nor any interaction of drug×order (all p>.40); thus, this variable was dropped from further analyses. Repeated-measures ANOVA of the mean amplitude of PA trials from the first block revealed that there was neither a main effect of drug ($F(1,49)=1.53$, p=.22, $\eta_p^2=.03$) nor an interaction effect of drug×genotype ($F(2,49)=0.72$, p=.49, $\eta_p^2=.03$). A similar analysis of the mean amplitude of all PA trials showed that there was neither a main effect of drug ($F(1,49)=2.49$, p=.12, $\eta_p^2=.05$) nor an interaction effect of drug×genotype ($F(2,49)=1.03$, p=.37, $\eta_p^2=.04$).

Repeated-measures ANOVA of habituation data (early habituation, total habituation, and linear gradient coefficient *b*) demonstrated that for early habituation, there was neither a

main effect of drug ($F(1,49)=0.75$, $p=.39$, $\eta_p^2=.02$) nor an interaction effect of drug×genotype ($F(2,49)=0.29$, $p=.75$, $\eta_p^2=.01$). However, there was a significant main effect of drug ($F(1,49)=5.59$, $p=.02$, $\eta_p^2=.10$) on total habituation indicating less habituation under nicotine as compared to placebo (see Table 3). No significant interaction of drug×genotype on total habituation was found ($F(2,49)=1.32$, $p=.28$, $\eta_p^2=.05$). Regarding linear gradient coefficient *b*, neither a significant main effect of drug ($F(1,49)=2.78$, $p=.10$, $\eta_p^2=.05$), nor a significant interaction of drug×genotype was found ($F(2,49)=0.24$, $p=.79$, $\eta_p^2=.01$).

(Table 3 about here)

Effects of nicotine on prepulse inhibition

Repeated-measures ANCOVA of PPI data revealed that there was neither a main effect nor any interaction effects of order (all $p>.44$); thus this variable was dropped from further analyses. There was no main effect of drug ($F(1,46)=0.20$, $p=.66$, $\eta_p^2=.004$), however, there was a significant interaction of drug×genotype ($F(2,46)=3.83$, $p=.03$, $\eta_p^2=.14$) indicating that the effect of nicotine on mean PPI across SOAs was modulated by *CHRNA3* rs1051730 genotype. Post-hoc testing revealed that TT-genotype-carriers of *CHRNA3* rs1051730 exhibited increased PPI under nicotine as compared to placebo ($p=.03$) whereas there was a trend for nicotine decreasing PPI in TC-carriers as compared to placebo ($p=.16$). The effect of nicotine in CC-carriers was similar to the one in TC-carriers: nicotine tended to decrease PPI as compared to placebo, although this effect was not statistically significant ($p=.28$) (see Figure 2).

(Figure 2 about here)

There was also significant triple interaction of drug×genotype×SOA ($F(6,138)=2.43$, $p=.04$, $\eta_p^2=.10$) indicating the interaction of drug×genotype was modulated by SOA. Post-hoc

testing revealed that in TT-carriers and TC-carriers the drug-by-genotype interaction was significant for the SOAs 120ms and 240ms, but not for the SOAs 30ms and 60ms (see Figure 3). In CC-carriers pattern of results was similar, but not statistically significant. We also found a main effect of SOA ($F(3,138)=3.78$, $p=.03$, $\eta_p^2=.20$), replicating the well-known nature of PPI to increase with rising SOA from 30ms to 120ms and to decrease from SOA 240ms onwards (Blumenthal 1999). No significant interaction of SOA×genotype ($F(1,46)=1.38$, $p=.25$, $\eta_p^2=.05$), and no significant interaction of SOA×drug ($F(1,46)=0.15$, $p=.90$, $\eta_p^2=.003$) was found. As startle reactivity (assessed by the mean amplitude of the first block of PA trials) tended to be different between genotype groups ($p=.07$, see Table 2) we added startle reactivity as an additional covariate to the statistical design (Csomor et al. 2008). Adding startle reactivity as an additional covariate to the statistical design did not change results regarding the lack of main effects of drug or genotype. Likewise, all interaction effects were not substantially altered by startle reactivity. The interaction of drug×genotype remained significant ($F(2,45)=3.93$, $p=.03$, $\eta_p^2=.14$) and the triple interaction of drug×genotype×SOA also survived ($F(6,135)=2.42$, $p=.04$, $\eta_p^2=.10$).

(Figure 3 about here)

Cotinine plasma levels

Cotinine data of 51 participants were available. Mean cotinine plasma levels were significantly higher for the nicotine session (mean cotinine=16.48±2.68 ng/ml) than for the placebo session ($t(50)=9.48$, $p<.1\times10^{-30}$) indicating a successful experimental manipulation. In total, PPI change scores were not significantly correlated with cotinine plasma level but in TC-carriers, PPI change scores tended to correlate with cotinine levels ($r=.36$, $p=.09$) indicating that with nicotine-induced decrease of PPI were associated with increased cotinine plasma level.

Discussion

The present study demonstrated that the effect of nicotine on PPI is modulated by a single nucleotide polymorphism in the cholinergic receptor gene *CHRNA3*. Nicotine significantly enhanced PPI in rs1051730 TT-homozygotes only, who displayed the lowest PPI levels, and tended to worsen PPI in TC- and CC-carriers, who showed normal PPI levels. In contrast, nicotine did not influence startle reactivity but significantly reduced total habituation of pulse-alone trials independent of genotype.

To our knowledge, this is the first pharmacogenetic study investigating the impact of a cholinergic receptor gene on the effect of nicotine on PPI. Our finding suggest that experiencing neurocognitive enhancement from nicotine application might represent another contributing factor in the development and maintenance of nicotine addiction – in line with the previously reported self-medication hypothesis (Kumari and Postma 2005). It remains unclear, however, which mechanisms are responsible for the beneficial effect of nicotine on PPI exclusively in TT-carriers. Possibly, an individual being TT-homozygous for the SNP rs1051730 is more susceptible for positive effects of nicotine (i.e., neurocognitive enhancement, elevated mood, etc.) and thereby more prone to develop and maintain nicotine dependence. Another, not mutually exclusive, possibility is that individuals with one or more copies of the T-allele may be more resistant to the aversive side effects of nicotine (Munafo et al. 2011). Interestingly, it is the T-allele of the rs1051730 polymorphism which has been repeatedly associated with nicotine dependence (Berrettini et al. 2008; Bierut et al. 2008; Caporaso et al. 2009; Saccone et al. 2009). Moreover, there is evidence for an association between the T-allele of the rs1051730 polymorphism and decreased likelihood of smoking cessation (Munafo et al. 2011) – further emphasizing the role of the T-allele in the context of nicotine dependence and smoking behavior.

PPI is classified as an established endophenotype or biomarker of schizophrenia (Braff et al. 2001). Therefore, it would also be interesting to investigate whether the present

pharmacogenetic interaction of a cholinergic SNP and nicotine on PPI can be found in schizophrenia patients. The T-allele of the rs1051730 polymorphism is not known to be a risk allele for schizophrenia, however, in our previous study we demonstrated an association with chronicity, treatment, and negative symptoms in our schizophrenia sample (Petrovsky et al. 2010). To date, there is no published study investigating the pharmacogenetic interaction of a cholinergic polymorphism with nicotine on neurocognition in schizophrenia. So far, there is only one published study providing direct molecular genetic evidence for the smoking-schizophrenia comorbidity (Hong et al. 2011b). That study revealed that the *CHRNA5* SNP rs16969968 (Asp398Asn) was simultaneously linked to smoking and schizophrenia in the same cohort (Hong et al. 2011b). Thus, it remains to be investigated whether genetic variants within the *CHRNA3/CHRNA5* gene cluster modulate the effects of nicotine on schizophrenia endophenotypes in patient samples.

An important point is that in the present investigation, we tested the effect of acute nicotine in non-smokers (individuals relatively naïve to nicotine): it is possible that in smokers, the modulating gene effect on the response to nicotine might be different. In a recent study, schizophrenia risk polymorphisms in the *TCF4* gene interacted with smoking in the modulation of P50 sensory gating (Quednow et al. 2012). In that study, only smokers reliably showed *TCF4*-P50 suppression associations, whereas the genetic effect was small or not present in never-smokers. Moreover, genotype-smoking interactions were dose-related, because *TCF4* SNP genotype effects amplified with increasing smoking severity. The results from that study might represent an example of a gene-environment interaction, in which smoking represents a long-lasting and ongoing environmental influence (Quednow et al. 2012). Thus, it would be of interest to investigate pharmacogenetic effects on gating mechanisms in smokers to further disentangle the causal relationships between diminished gating performance, nicotine sensitivity, nicotine intake, and genetic variation.

Limitations of the present study include the relatively small sample size of the genotype groups. A related issue involves the non-significant main effect of genotype on PPI (i.e. significantly diminished PPI in TT-carriers) as in our previous study (Petrovsky et al. 2010). However, numerically the TT-genotype group did show the smallest PPI with considerable effect size. Presumably, our sample was not large enough in order to significantly replicate our previous association as the present study's sample was much smaller than the prior one. Therefore, the present results should be regarded as preliminary requiring replication in a larger sample.

In conclusion, we demonstrated that the effect of nicotine on sensorimotor gating is modulated by a *CHRNA3* polymorphism confirming a major role of α3-subunits of the nAChR in the mediation of PPI. We showed that carriers of a risk allele for nicotine dependence are also those individuals whose PPI is enhanced by nicotine. Thus, genetic variation in cholinergic genes might be an important connecting link between cognitive deficits and smoking behavior. Finally, α3-subunits might represent new targets for developing pharmacological treatment of cognitive dysfunction in schizophrenia and in other disorders.

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Conflict of Interest

All authors declare no conflict of interest.

References

Acri JB, Brown KJ, Saah MI, Grunberg NE (1995) Strain and age differences in acoustic startle responses and effects of nicotine in rats. Pharmacology, biochemistry, and behavior 50: 191-8.

Acri JB, Morse DE, Popke EJ, Grunberg NE (1994) Nicotine increases sensory gating measured as inhibition of the acoustic startle reflex in rats. Psychopharmacology 114: 369-74.

Baschnagel JS, Hawk LW, Jr. (2008) The effects of nicotine on the attentional modification of the acoustic startle response in nonsmokers. Psychopharmacology 198: 93-101.

Berrettini W, Yuan X, Tozzi F, Song K, Francks C, Chilcoat H, Waterworth D, Muglia P, Mooser V (2008) Alpha-5/alpha-3 nicotinic receptor subunit alleles increase risk for heavy smoking. Molecular psychiatry 13: 368-73.

Bierut LJ, Stitzel JA, Wang JC, Hinrichs AL, Grucza RA, Xuei X, Saccone NL, Saccone SF, Bertelsen S, Fox L, Horton WJ, Breslau N, Budde J, Cloninger CR, Dick DM, Foroud T, Hatsukami D, Hesselbrock V, Johnson EO, Kramer J, Kuperman S, Madden PA, Mayo K, Nurnberger J, Jr., Pomerleau O, Porjesz B, Reyes O, Schuckit M, Swan G, Tischfield JA, Edenberg HJ, Rice JP, Goate AM (2008) Variants in nicotinic receptors and risk for nicotine dependence. The American journal of psychiatry 165: 1163-71.

Blumenthal TD (1999) Short lead interval startle modification. In: Dawson ME, Schell AM, Böhmelt AH (eds) Startle Modification Implications for Neuroscience, Cognitive Sciences, and Clinical Science. Cambridge University Press, Cambridge, pp 51-71

Blumenthal TD, Cuthbert BN, Filion DL, Hackley S, Lipp OV, van Boxtel A (2005) Committee report: Guidelines for human startle eyeblink electromyographic studies. Psychophysiology 42: 1-15.

Bosch D, Schmid S (2008) Cholinergic mechanism underlying prepulse inhibition of the startle response in rats. Neuroscience 155: 326-35.

Braff DL, Geyer MA, Swerdlow NR (2001) Human studies of prepulse inhibition of startle: normal subjects, patient groups, and pharmacological studies. Psychopharmacology 156: 234-58.

Caporaso N, Gu F, Chatterjee N, Sheng-Chih J, Yu K, Yeager M, Chen C, Jacobs K, Wheeler W, Landi MT, Ziegler RG, Hunter DJ, Chanock S, Hankinson S, Kraft P, Bergen AW (2009) Genome-wide and candidate gene association study of cigarette smoking behaviors. PloS one 4: e4653.

Csomor PA, Yee BK, Vollenweider FX, Feldon J, Nicolet T, Quednow BB (2008) On the influence of baseline startle reactivity on the indexation of prepulse inhibition. Behavioral neuroscience 122: 885-900.

Curzon P, Kim DJ, Decker MW (1994) Effect of nicotine, lobeline, and mecamylamine on sensory gating in the rat. Pharmacology, biochemistry, and behavior 49: 877-82.

Della Casa V, Hofer I, Weiner I, Feldon J (1998) The effects of smoking on acoustic prepulse inhibition in healthy men and women. Psychopharmacology 137: 362-8.

Depatie L, O'Driscoll GA, Holahan AL, Atkinson V, Thavundayil JX, Kin NN, Lal S (2002) Nicotine and behavioral markers of risk for schizophrenia: a double-blind, placebo-controlled, cross-over study. Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology 27: 1056-70.

Duncan E, Madonick S, Chakravorty S, Parwani A, Szilagyi S, Efferen T, Gonzenbach S, Angrist B, Rotrosen J (2001) Effects of smoking on acoustic startle and prepulse inhibition in humans. Psychopharmacology 156: 266-72.

Faraday MM, O'Donoghue VA, Grunberg NE (1999) Effects of nicotine and stress on startle amplitude and sensory gating depend on rat strain and sex. Pharmacology, biochemistry, and behavior 62: 273-84.

Gabr RQ, Elsherbiny ME, Somayaji V, Pollak PT, Brocks DR (2011) A liquid chromatography-mass spectrometry method for nicotine and cotinine; utility in screening tobacco exposure in patients taking amiodarone. Biomedical chromatography : BMC 25: 1124-31.

Graham FK (1975) Presidential Address, 1974. The more or less startling effects of weak prestimulation. Psychophysiology 12: 238-48.

Heishman SJ, Kleykamp BA, Singleton EG (2010) Meta-analysis of the acute effects of nicotine and smoking on human performance. Psychopharmacology 210: 453-69.

Hong LE, Thaker GK, McMahon RP, Summerfelt A, Rachbeisel J, Fuller RL, Wonodi I, Buchanan RW, Myers C, Heishman SJ, Yang J, Nye A (2011a) Effects of moderate-dose treatment with varenicline on neurobiological and cognitive biomarkers in smokers and nonsmokers with schizophrenia or schizoaffective disorder. Archives of general psychiatry 68: 1195-206.

Hong LE, Wonodi I, Lewis J, Thaker GK (2008) Nicotine effect on prepulse inhibition and prepulse facilitation in schizophrenia patients. Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology 33: 2167-74.

Hong LE, Yang X, Wonodi I, Hodgkinson CA, Goldman D, Stine OC, Stein ES, Thaker GK (2011b) A CHRNA5 allele related to nicotine addiction and schizophrenia. Genes, brain, and behavior 10: 530-5.

Kumari V, Checkley SA, Gray JA (1996) Effect of cigarette smoking on prepulse inhibition of the acoustic startle reflex in healthy male smokers. Psychopharmacology 128: 54-60.

Kumari V, Cotter PA, Checkley SA, Gray JA (1997) Effect of acute subcutaneous nicotine on prepulse inhibition of the acoustic startle reflex in healthy male non-smokers. Psychopharmacology 132: 389-95.

Kumari V, Postma P (2005) Nicotine use in schizophrenia: the self medication hypotheses. Neuroscience and biobehavioral reviews 29: 1021-34.

Levin ED, Conners CK, Silva D, Hinton SC, Meck WH, March J, Rose JE (1998) Transdermal nicotine effects on attention. Psychopharmacology 140: 135-41.

Munafo MR, Johnstone EC, Walther D, Uhl GR, Murphy MF, Aveyard P (2011) CHRNA3 rs1051730 genotype and short-term smoking cessation. Nicotine & tobacco research : official journal of the Society for Research on Nicotine and Tobacco 13: 982-8.

Perkins KA, Lerman C, Coddington S, Jetton C, Karelitz JL, Wilson A, Jennings JR, Ferrell R, Bergen AW, Benowitz NL (2008) Gene and gene by sex associations with initial sensitivity to nicotine in nonsmokers. Behavioural pharmacology 19: 630-40.

Petrovsky N, Ettinger U, Quednow BB, Walter H, Schnell K, Kessler H, Mossner R, Maier W, Wagner M (2012) Nicotine differentially modulates antisaccade performance in healthy male non-smoking volunteers stratified for low and high accuracy. Psychopharmacology 221: 27-38.

Petrovsky N, Quednow BB, Ettinger U, Schmechtig A, Mossner R, Collier DA, Kuhn KU, Maier W, Wagner M, Kumari V (2010) Sensorimotor gating is associated with CHRNA3 polymorphisms in schizophrenia and healthy volunteers. Neuropsychopharmacology 35: 1429-39.

Poltavski DV, Petros T (2006) Effects of transdermal nicotine on attention in adult non-smokers with and without attentional deficits. Physiology & behavior 87: 614-24.

Quednow BB, Brinkmeyer J, Mobascher A, Nothnagel M, Musso F, Grunder G, Savary N, Petrovsky N, Frommann I, Lennertz L, Spreckelmeyer KN, Wienker TF, Dahmen N, Thuerauf N, Clepce M, Kiefer F, Majic T, Mossner R, Maier W, Gallinat J, Diaz-Lacava A, Toliat MR, Thiele H, Nurnberg P, Wagner M, Winterer G (2012) Schizophrenia risk polymorphisms in the TCF4 gene interact with smoking in the

modulation of auditory sensory gating. Proceedings of the National Academy of Sciences of the United States of America 109: 6271-6.

Quednow BB, Kuhn KU, Beckmann K, Westheide J, Maier W, Wagner M (2006a) Attenuation of the prepulse inhibition of the acoustic startle response within and between sessions. Biological psychology 71: 256-63.

Quednow BB, Wagner M, Westheide J, Beckmann K, Bliesener N, Maier W, Kuhn KU (2006b) Sensorimotor gating and habituation of the startle response in schizophrenic patients randomly treated with amisulpride or olanzapine. Biological psychiatry 59: 536-45.

Saccone NL, Saccone SF, Hinrichs AL, Stitzel JA, Duan W, Pergadia ML, Agrawal A, Breslau N, Grucza RA, Hatsukami D, Johnson EO, Madden PA, Swan GE, Wang JC, Goate AM, Rice JP, Bierut LJ (2009) Multiple distinct risk loci for nicotine dependence identified by dense coverage of the complete family of nicotinic receptor subunit (CHRN) genes. American journal of medical genetics Part B, Neuropsychiatric genetics : the official publication of the International Society of Psychiatric Genetics 150B: 453-66.

Schreiber R, Dalmus M, De Vry J (2002) Effects of alpha 4/beta 2- and alpha 7-nicotine acetylcholine receptor agonists on prepulse inhibition of the acoustic startle response in rats and mice. Psychopharmacology 159: 248-57.

Spielewoy C, Markou A (2004) Strain-specificity in nicotine attenuation of phencyclidine-induced disruption of prepulse inhibition in mice: relevance to smoking in schizophrenia patients. Behavior genetics 34: 343-54.

Swerdlow NR, Hartman PL, Auerbach PP (1997) Changes in sensorimotor inhibition across the menstrual cycle: implications for neuropsychiatric disorders. Biological psychiatry 41: 452-60.

Wittchen H-U, Wunderlich U, Gruschwitz S, Zaudig M (1997) SKID-I. Strukturiertes Klinisches Interview für DSM-IV. Achse I: Psychische Störungen. Interviewheft. (SCID-I. Structured Clinical Interview for DSM-IV, German version). Göttingen: Hogrefe

Tables

Table 1 Experimental procedure of the two testing sessions.

Before patch application:	Visit study physician and informed consent (at session 1) ($t_{-60\ min}$) Urine drug screening test (additionally in females: urine pregnancy test)($t_{-45\ min}$)
Patch application	Administration of 7mg nicotine or placebo (t_0)
3-hour-waiting time:	SCID-I interview ($t_{+5\ min}$) Verbal IQ testing ($t_{+60\ min}$) Collect demographic data ($t_{+70\ min}$) Participants were allowed to read and drink water ($t_{+90\text{-}160\ min}$) Application of the electrodes ($t_{+160\ min}$)
3 hours after patch application:	Acoustic startle measurement ($t_{+180\ min}$) Taking of blood sample for cotinine plasma levels and genotyping ($t_{+200\ min}$) Patch removal ($t_{+215\ min}$) Debriefing and financial compensation (at session 2)

Table 2 Demographic characteristics and psychophysiological parameters of participants.

***CHRNA3* rs1051730 genotype**	**TT**	**TC**	**CC**	**Total**	**F/χ^2**	**df/df_{err}**	**p**	**η_p^2**
N	10 (19.2%)	24 (46.2%)	18 (34.6%)	52 (100%)				
Age (years)	32.00 (13.08)	27.75 (6.33)	27.83 (8.20)	28.60 (8.57)	0.98	2/49	.38	.04
Years of education	16.20 (2.25)	16.33 (0.87)	16.44 (1.98)	16.35 (1.60)	0.74	2/49	.93	.003
Body mass index (BMI)	23.81 (4.55)	23.30 (3.52)	24.33 (3.64)	23.76 (3.72)	0.38	2/49	.69	.02
Men (%)	40.0	54.2	77.8	59.6	4.36	2	.11	-
Placebo condition: First block, amplitude of pulse-alone trials (arbitrary units)	241.82 (175.78)	290.89 (239.63)	447.52 (293.12)	335.67 (258.99)	2.89	2/49	.07	.11
Placebo condition: Mean amplitude of pulse-alone trials (arbitrary units)	185.63 (148.44)	215.89 (212.73)	317.71 (259.81)	245.32 (223.06)	1.55	2/49	.22	.06
Placebo condition: Mean percent prepulse inhibition (overall PPI, averaged over all SOAs)	33.50 (25.85)	37.58 (28.08)	47.66 (22.66)	40.29 (26.01)	1.20	2/49	.31	.05
Placebo condition: Percent early habituation of pulse-alone trials (between first and second block)	25.38 (31.41)	29.47 (22.67)	34.85 (22.29)	30.55 (24.16)	0.53	2/49	.59	.02
Placebo condition: Percent total habituation of pulse-alone trials (between first and fourth block)	37.31 (26.10)	47.38 (24.90)	52.71 (24.16)	47.29 (24.99)	1.23	2/49	.30	.05
Placebo condition: Habituation of pulse-alone trials across 4 blocks (linear gradient coefficient *b*)	-29.24 (26.32)	-39.78 (31.46)	-67.46 (45.82)	-47.33 (38.76)	4.52	2/49	.02[a]	.16

Legend. Data represent means (standard deviations) unless otherwise specified.
[a]Bonferroni post-hoc tests: TT > TC p=1.00, TT > CC p=.03, TC > CC p=.055

Table 3 The effect of nicotine on startle reactivity, prepulse inhibition and habituation.

***CHRNA3* rs1051730 genotype**	**TT**		**TC**		**CC**		**Total**		**Significant main effect of drug?**	**Significant interaction of drug×genotype?**
	Placebo	**Nicotine**	**Placebo**	**Nicotine**	**Placebo**	**Nicotine**	**Placebo**	**Nicotine**		
First block, amplitude of pulse-alone trials (arbitrary units)	241.82 (175.78)	239.39 (223.48)	290.89 (239.63)	276.75 (216.89)	447.52 (293.12)	391.33 (320.69)	335.67 (258.99)	309.22 (261.04)	No	No
Mean amplitude of pulse-alone trials (arbitrary units)	185.63 (148.44)	180.12 (187.60)	215.89 (212.73)	205.43 (186.37)	317.71 (259.81)	272.47 (247.87)	245.32 (223.06)	223.77 (209.10)	No	No
Mean percent prepulse inhibition (overall PPI, averaged over all SOAs)	33.50 (25.85)	43.58 (16.95)	37.58 (28.08)	33.75 (28.99)	47.66 (22.66)	43.63 (25.91)	40.29 (26.01)	39.06 (26.04)	No	Yes[a]
Percent early habituation of pulse-alone trials (between first and second block)	25.38 (31.41)	28.23 (26.74)	29.47 (22.67)	30.18 (28.97)	34.85 (22.29)	42.37 (19.74)	30.55 (24.16)	34.02 (25.94)	No	No
Percent total habituation of pulse-alone trials (between first and fourth block)	37.31 (26.10)	17.95 (55.15)	47.38 (24.90)	36.30 (30.63)	52.71 (24.16)	51.80 (27.45)	47.29 (24.99)	38.14 (36.86)	Yes[b]	No
Habituation of pulse-alone trials across 4 blocks (linear gradient coefficient *b*)	-29.24 (26.32)	-24.68 (29.12)	-39.78 (31.46)	-31.17 (29.47)	-67.46 (45.82)	-53.39 (41.85)	-47.33 (38.76)	-37.61 (35.57)	No	No

Legend. Data represent means (standard deviations). Nicotine=nic, placebo=plac.
[a]Interaction of drug×genotype ($F(2,46)=3.83$, $p=.03$, $\eta_p^2=.14$). Bonferroni post-hoc tests: TT: nic>plac $p=.03$, TC: nic<plac $p=.16$, CC: n<plac $p=.28$.
[b]Main effect of drug ($F(1,49)=5.59$, $p=.02$, $\eta_p^2=.10$) indicating less habituation under nicotine as compared to placebo.

Figure legends

Figure 1 Placebo condition: habituation curves diagrammed as mean amplitude of pulse-alone (PA) trials in 4 blocks across *CHRNA3* rs1051730 genotype groups (means±SEM).

Legend. Genotype groups differed significantly in habituation of pulse-alone trials across all four blocks (linear coefficient b)(p=.02). Post-hoc testing revealed that there was no difference between TT-carriers and TC-carriers (p=1.00). TT-carriers exhibited a decreased habituation coefficient compared to CC-carriers (p=.03). TC-carriers tended to show a decreased habituation coefficient compared to CC-carriers (p=.055).

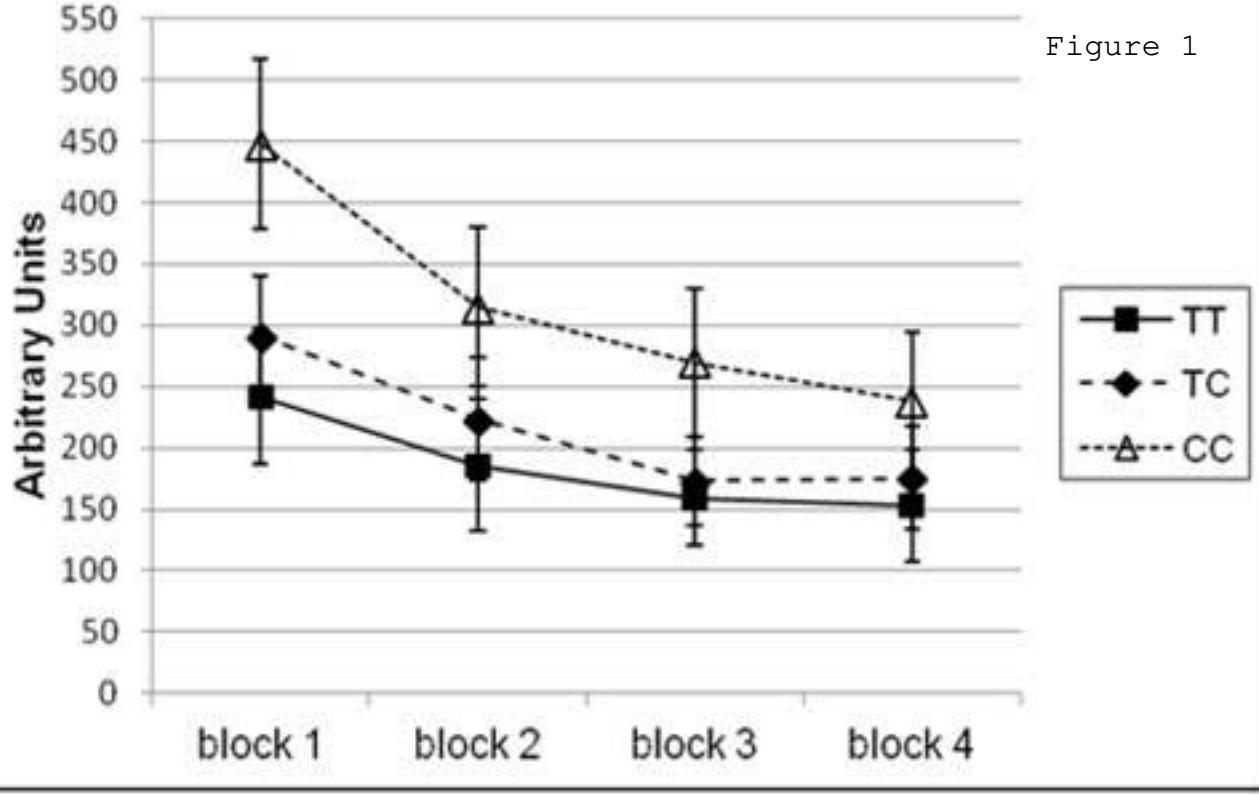
Figure 2 The effect of nicotine on mean PPI across SOAs is modulated by *CHRNA3* rs1051730 genotype in 52 healthy non-smoking human volunteers (means±SEM, adjusted for sex).

Legend. There was a significant interaction of drug × genotype (p=.03). Post-hoc testing revealed that nicotine enhanced PPI in *CHRNA3* rs1051730 TT-carriers (p=.03) while nicotine non-significantly tended to worsen PPI in TC-carriers (p=.06) and CC-carriers (p=.13).

Figure 3 The effects of nicotine on mean PPI for each SOA and *CHRNA3* rs1051730 genotype (means±SEM, adjusted for sex).

Legend. There was a significant triple interaction of drug × genotype × SOA (p=.04) indicating the interaction of drug × genotype was modulated by SOA. Post-hoc testing showed that the interaction effect was more pronounced for SOAs 120 ms and 240 ms than for SOAs 30 ms and 60 ms.

Figure 1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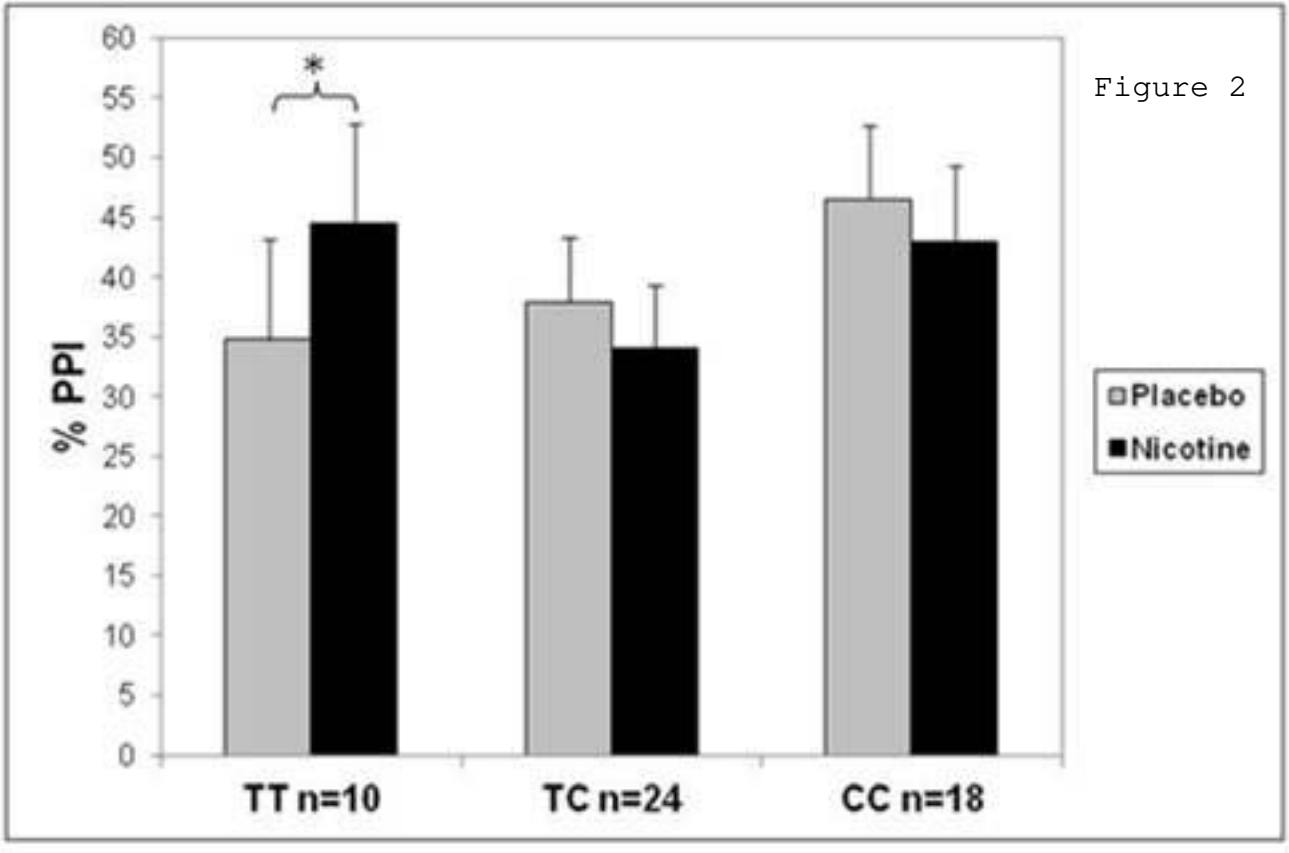
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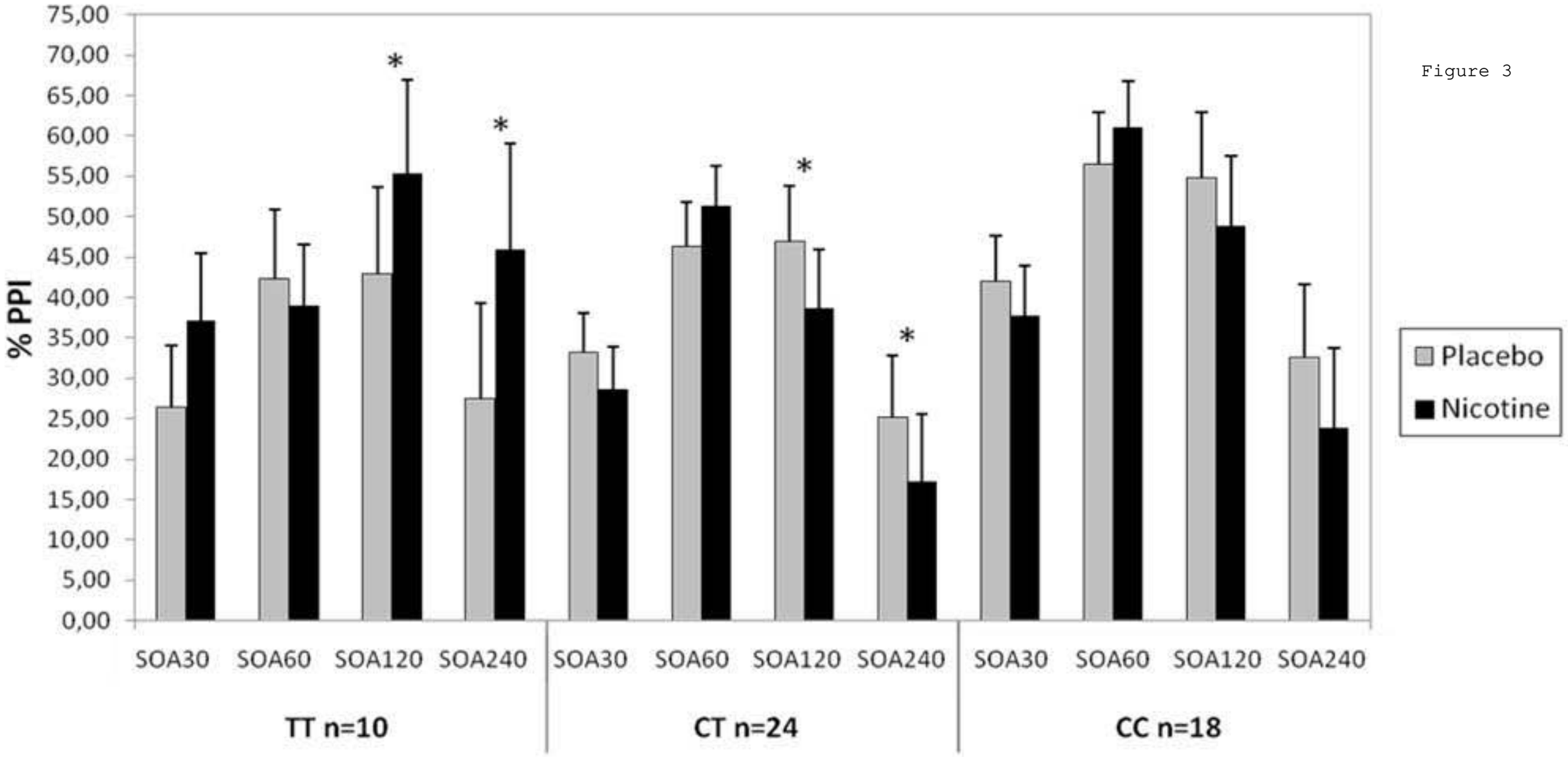
Figure 2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TT n=10
CT n=24
CC n=18
Placebo
Nicotine

Figure 3